

**Data Evaluation Report on the Acute Toxicity of Aminocyclopyrachlor acid technical to Algae (*Pseudokirchneriella subcapitata*)**

PMRA Submission Number {.....}

EPA MRID Number 47560203

**Data Requirement:**

PMRA DATA CODE	{.....}
EPA DP Barcode	358148
OECD Data Point	{.....}
EPA MRID	47560203
EPA Guideline	OPPTS 850.5400 (158.660)

**Test material:** DPX-MAT 28 (Aminocyclopyrachlor acid) technical **Purity:** 92.2%

Common name

Chemical name:

IUPAC 6-Amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid

CAS name 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

6-Amino-5-chloro-2-cyclopropyl-pyrimidine-4-carboxylic acid

CAS No. 858956-08-8

Synonyms DPX-MAT28, Aminocyclopyrachlor

**Primary Reviewer:** Moncie Wright  
**Staff Scientist, Cambridge Environmental****Signature:****Date:** 6/10/09**Primary Reviewer:** Teri Myers  
**Senior Scientist, Cambridge Environmental****Signature:****Date:** 7/10/09**Secondary Reviewer:** Anita Ullagaddi  
**EPA/OPP/EFED/ERB1****Signature:****Date:** 10/08/09**Reference/Submission No.:** {.....}**Company Code** {.....} [For PMRA]**Active Code** {.....} [For PMRA]**Use Site Category:** {.....} [For PMRA]**EPA PC Code** None**Date Evaluation Completed:** 10/08/09**CITATION:** Porch, J.R., Kendall, T.Z., and H.O. Krueger. 2008. DPX-MAT28 Technical: A 72-Hour Toxicity Test with the Freshwater Algae (*Pseudokirchneriella subcapitata*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No.: 112A-217A. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, Delaware. Study completed May 7, 2008 and revised June 2, 2008.**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of

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factors related to the test methodology and results in determining the acceptability of the study.

## **EXECUTIVE SUMMARY:**

In a 72-hour acute toxicity study, cultures of the freshwater green algae (*Pseudokirchneriella subcapitata*) were exposed to Aminocyclopyrachlor acid technical at nominal concentrations of 0 (negative control), 7.5, 15, 30, 60, and 120 mg ai/L under static conditions. Mean-measured concentrations were <0.0165 (<LOD, control), 7.35, 15.3, 30.1, 59.1, and 120 mg ai/L.

Inhibitions for cell density ranged from 5 to 48% in the treatment groups as compared to the controls. Inhibition for biomass ranged from 6 to 40%. Growth rate had the lowest inhibitions, which ranged from 1 to 12%.

No endpoint exhibited inhibition in excess of 50%; the EC<sub>50</sub> value for all endpoints was >120 mg ai/L. The NOAEC for biomass and cell density was determined to be 15.3 mg a.i./L and the NOAEC for growth rate was 59.1 mg ai/L. In addition, cell density and biomass shared the lower EC<sub>05</sub> estimate of 62 mg ai/L while the growth rate estimate was 93 mg a.i./L.

Cell morphology in the control and treatment groups was normal in size, shape, and color. There was no evidence of flocculation, aggregation, or adherence to the test chambers in any group.

This toxicity study is scientifically sound and classified as acceptable. It satisfies the guideline requirement for a Tier I screening nonvascular aquatic plant toxicity study with the freshwater green algae, *Pseudokirchneriella subcapitata*.

## **Results Synopsis**

Test Organism: *Pseudokirchneriella subcapitata*

Test Type (Flow-through, Static, Static Renewal): Static

### **Cell Density**

EC<sub>05</sub>: 62 mg ai/L 95% C.I.: 41 to 94 mg ai/L

EC<sub>50</sub>: >120 mg ai/L 95% C.I.: N/A

NOAEC: 15.3 mg ai/L

Probit Slope: 5.35 ± 1.60

### **Biomass (Area Under the Growth Curve)**

EC<sub>05</sub>: 62 mg ai/L 95% C.I.: 39 to 100 mg ai/L

EC<sub>50</sub>: >120 mg ai/L 95% C.I.: N/A

NOAEC: 15.3 mg ai/L

Probit Slope: 4.58 ± 1.54

### **Growth Rate**

EC<sub>05</sub>: 93 mg ai/L 95% C.I.: 77 to 110 mg ai/L

EC<sub>50</sub>: >120 mg ai/L 95% C.I.: N/A

NOAEC: 59.1 mg ai/L

Probit Slope: 3.98 ± 1.35

Endpoint(s) Effected: Cell density, biomass, and growth rate

## **I. MATERIALS AND METHODS**

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**GUIDELINE FOLLOWED:**

This study was conducted following OECD Guideline for Testing of Chemicals, 201: *Alga, Growth Inhibition Test*, and U.S. EPA Series 850 – Ecological Effects Test Guidelines, OPPTS Guideline 850.5400: *Algal Toxicity, Tiers I and II*. The following deviations from OPPTS 850.5400 were noted:

1. Total organic carbon, particulate matter, and chlorine concentrations of the well water used for the algal medium were not reported.
2. The initial pH of the 59.1 and 120 mg ai/L test levels (7.0 and 6.1, respectively) was lower than recommended by OPPTS guidelines at test initiation (i.e.,  $7.5 \pm 0.1$ ) and the initial pH of the lowest test level (i.e., 7.9) was higher than recommended.
3. The physico-chemical properties of the test material were not reported.
4. The study was only conducted for 72 hours; OPPTS Guidelines suggest a minimum study period of 96 hours. However, 72 hours is acceptable by OECD guidelines. According to an EPA memo (October 1994) entitled, "Closure on Nontarget Plant Phytotoxicity Policy Issues", 3-day OECD studies should be considered for Tier I screening purposes only.

These deviations do not affect the acceptability of this study.

**COMPLIANCE:**

Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with U.S. EPA FIFRA GLP standards (40 CFR Parts 160 and 792), that are consistent with OECD Principles of GLP (ENV/MC/CHEM(98)17), and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1999), with the following exception:  
Periodic analyses of well water for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods.

**A. MATERIALS:**

**1. Test material**

DPX-MAT28 (Aminocyclopyrachlor acid) technical

**Description:**

Solid

**Lot No./Batch No. :**

DPX-MAT28-009 (Batch No.)

**Purity:**

92.2%

**Stability of compound under test conditions:**

The day 0 measured concentrations yielded recoveries of 98% to 102% of nominal test concentrations, and day 3 measured concentrations yielded recoveries of 97% to 101% of nominal. Aminocyclopyrachlor acid technical was very stable under the test conditions.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

**Storage conditions of test chemicals:**

Test material was stored at ambient temperature.

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## Physicochemical properties of Aminocyclopyrachlor acid technical.

Parameter	Values	Comments
Water solubility at 20EC	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

## 2. Test organism:

**Name:** Green algae, *Pseudokirchneriella subcapitata*  
*EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricornutum, and a freshwater diatom is tested.*

*OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported*

**Strain:** Not reported  
**Source:** In-house cultures originally obtained from the University of Toronto, Canada  
**Age of inoculum:** At least 2 weeks prior to test initiation  
**Method of cultivation:** Cultured and tested in OECD freshwater algal medium

## B. STUDY DESIGN:

### 1. Experimental Conditions

- a. Range-finding study A range-finding study was conducted, but the details were not reported.
- b. Definitive Study

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
Acclimation period:	Continuous	
Culturing media and conditions:	OECD freshwater medium	

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Parameter	Details	Remarks
		Criteria
(same as test or not)	Could not be determined.	<i>EPA recommends two week acclimation period.</i>
Health: (any mortality observed)	Algal cells appeared normal at test initiation.	<i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i>
<u>Test system</u> Static/static renewal	Static	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Renewal rate for static renewal	N/A	
Incubation facility	Test vessels were placed on a mechanical shaker in an environmental chamber.	
Duration of the test	72 hours	<i>EPA requires: 96-120 hours OECD: 72 hours</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Sterile Erlenmeyer flasks 250 mL 100 mL	Flasks were plugged with foam stoppers.  <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
<u>Details of growth medium name</u>		

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Parameter	Details	Remarks
		Criteria
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	6.1-7.9 6.0-8.2 Yes. NaHCO <sub>3</sub> N/A	OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.  EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
<u>Dilution water</u> source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Wildlife International well water Adjusted to 8.1 N/A Water was purified Not reported. Not reported. ND - 34.9 mg/L Not detected. Not reported.	----- EPA pH: <i>Skeletonema costatum</i> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.  OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.
Indicate how the test material is added to the medium (added directly or used stock solution)	A stock solution of 120 mg ai/L was prepared as the highest test concentration. This highest concentration stock solution was then proportionally diluted with algal medium.	Test concentrations were adjusted to 100% active ingredient based upon the reported purity.

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Parameter	Details	Remarks
		Criteria
Aeration or agitation	Agitation, 100 rpm	
Initial cells density	1.0 x 10 <sup>4</sup> cells/mL	<p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>
<u>Number of replicates</u> Control: Solvent control: Treatments:	6 N/A 3	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</p> <p>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</p>
<u>Test concentrations</u>		

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Parameter	Details	Remarks
		Criteria
Nominal:	0 (negative control), 7.5, 15, 30, 60, and 120 mg ai/L	EPA requires at least 5 test concentrations in a geometric series with a ratio between 1.5 and 2.0.
Mean-measured:	<0.0165 (<LOD, control), 7.5, 15, 30, 59, and 120 mg ai/L	OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples of the test solutions collected at 0 and 72 hours, quality control samples, and calibration standards prepared at 0 and 96 hours were analyzed using HPLC with UV (220 nm) detection.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	23.4-23.9°C Continuous 5030-7100 lux Cool-white fluorescent lighting	EPA temperature: <i>Skeletonema</i> : 20°C, Others: 24-25°C; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <i>Anabaena</i> : 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)  OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.
<u>Reference chemical (if used)</u> name: concentrations:	N/A	
Other parameters, if any	None.	



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**2. Observations:**

**Table 2: Observation parameters**

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	-cell density -area under the growth curve (biomass) -growth rate	<i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i>
Measurement technique for cell density and other end points	Cell density was measured daily using an electronic particle counter (Coulter Electronics, Inc.). Growth rate and area under the growth curve were calculated using initial cell density and cell density at each time interval.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i>  <i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i>
Observation intervals	Every 24 hours.	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	N/A	
Indicate whether there was an exponential growth in the control	Yes, mean cell density in the negative control was $217 \times 10^4$ cells/mL at test termination.	<i>EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.</i>  <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes.	

**II. RESULTS and DISCUSSION:**

**A. INHIBITORY EFFECTS:**

After 72 hours of exposure, cell density averaged  $217 \times 10^4$  cells/mL in the negative control, yielding inhibitions of

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5, 6, 12, 8, and 48% as compared to the negative control in the mean-measured 7.35, 15.3, 30.1, 59.1, and 120 mg ai/L treatment groups, respectively. The 72-hour NOAEC and EC<sub>50</sub> values based on cell density were reported by the study authors to be 7.4 and >120 mg ai/L, respectively.

Growth rate averaged 0.0747 cells/mL/hour in the negative control, yielding inhibitions of 1, 1, 3, 2, and 12% as compared to the control. The 72-hour NOAEC and EC<sub>50</sub> values based on growth rate were reported by the study authors to be 7.4 and >120 mg ai/L, respectively.

Biomass (area under the growth curve) averaged  $3,506 \times 10^4$  in the negative control, yielding inhibitions of 7, 6, 12, 8, and 40% as compared to the negative control. The 72-hour NOAEC and EC<sub>50</sub> values based on biomass were reported by the study authors to be <7.4 and >120 mg ai/L, respectively.

The study authors reported that cell morphology in the control and treatment groups was normal in size, shape, and color. There was no evidence of flocculation, aggregation, or adherence to the test chambers in any group.

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**Table 3: Effect of Aminocyclopyrachlor acid technical on algal growth (*Pseudokirchneriella subcapitata*)**

Mean-Measured and (Nominal) Concentrations (mg ai/L)	Initial Cell density ( $\times 10^4$ cells/mL)	Mean Cell density ( $\times 10^4$ cells/mL) at			
		24 hours	48 hours	72 hours	
				Cell count	% inhibition
Negative control	1.0	4.1	35.8	217.5	N/A
7.35 (7.5)	1.0	3.7	32.2	206.2	5
15.3 (15)	1.0	3.8	33.8	205.3	6
30.1 (30)	1.0	3.8	31.8	190.7	12
59.1 (60)	1.0	4.3	32.6	200.1	8
120 (120)	1.0	3.7	29.7	113.6	48

N/A- Not Applicable

**Table 4: Effect of Aminocyclopyrachlor acid technical on algal growth (*Pseudokirchneriella subcapitata*)**

Mean-Measured and (Nominal) Concentrations (mg ai/L)	Initial Cell Density ( $\times 10^4$ cells/mL)	Mean Growth Rate (cells/mL/hour)		Mean Area Under the Growth Curve (Biomass) ( $\times 10^6$ )	
		0-72 Hours	% Inhibition	0-72 hours	% Inhibition
Negative control	1.0	0.0747	N/A	35.1	N/A
7.35 (7.5)	1.0	0.0740	1	32.8	7
15.3 (15)	1.0	0.0739	1	33.1	6
30.1 (30)	1.0	0.0729	3	30.8	12
59.1 (60)	1.0	0.0736	2	32.2	8
120 (120)	1.0	0.0657	12	21.0	40

N/A- Not Applicable

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**Table 5: Statistical endpoint values reported by the study authors.**

Statistical Endpoint	Cell Density	Biomass (Area under the Growth Curve)	Growth Rate
NOAEC or EC <sub>05</sub> (mg ai/L)	7.4	<7.4	7.4
LOAEC	15	7.4	15
IC <sub>50</sub> or EC <sub>50</sub> (mg ai/L) (95% C.I.)	>120 (N/A)	>120 (N/A)	>120 (N/A)
Other (EC <sub>10</sub> and EC <sub>90</sub> )	N/A	N/A	N/A
Reference chemical, if used IC <sub>50</sub> /EC <sub>50</sub>	N/A	N/A	N/A

ND – not determined

**B. REPORTED STATISTICS:**

Statistical analysis was performed for the endpoints of cell density, biomass (area under the growth curve) and growth rate. The data were tested for normality using Shapiro-Wilk's test ( $\alpha = 0.01$ ), and for homogeneity of variance using Levene's test ( $\alpha = 0.01$ ). Since the data demonstrated normality and homogeneity of variance, the treatment groups were compared to the control using ANOVA and Dunnett's test ( $\alpha = 0.05$ ). Due to high variability in the test, Dunnett's test was not considered to be sensitive for the NOAEC determination. Therefore, the results were analyzed using a Jonckheere-Terpstra trend test ( $\alpha = 0.05$ ). The results of the statistical analyses and an evaluation of the concentration-response pattern were used to determine the NOAEC and LOAEC relative to each parameter at 72 hours. The study authors used SAS version 8.2 for statistical analyses.

**C. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Replicate data for all endpoints were assessed to determine toxicity values. The reviewer tested the replicate data for normality using the Chi-square and Shapiro Wilk's tests and for homogeneity of variance using Hartley and Bartlett's test. If the assumptions of ANOVA were met, the NOAEC value was determined using the parametric Dunnett's and William's Test. If the assumptions were not met, the NOAEC value was determined using the non-parametric Steels or Kruskal-Wallis Test. All NOAEC values were determined using Toxstat Statistical Software. ECx values (with 95% C.I.) and probit slopes were determined using probit analyses via Nuthatch Statistical Software. All toxicity values were determined using the 72-hour mean-measured concentrations verified by the reviewer.

Cell density values were entered into Toxstat as the value  $\times 10^4$ . For biomass, data values were entered into Toxstat as the value  $\times 10^6$ . Growth rate data were multiplied by 10,000 before entry into Toxstat.

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**Cell Density**

EC <sub>05</sub> : 62 mg ai/L	95% C.I.: 41 to 94 mg ai/L
EC <sub>50</sub> : >120 mg ai/L	95% C.I.: N/A
NOAEC: 15.3 mg ai/L	
Probit Slope: 5.35 ± 1.60	

**Biomass (Area Under the Growth Curve)**

EC <sub>05</sub> : 62 mg ai/L	95% C.I.: 39 to 100 mg ai/L
EC <sub>50</sub> : >120 mg ai/L	95% C.I.: N/A
NOAEC: 15.3 mg ai/L	
Probit Slope: 4.58 ± 1.54	

**Growth Rate**

EC <sub>05</sub> : 93 mg ai/L	95% C.I.: 77 to 110 mg ai/L
EC <sub>50</sub> : >120 mg ai/L	95% C.I.: N/A
NOAEC: 15.3 mg ai/L	
Probit Slope: 3.98 ± 1.35	

**D. STUDY DEFICIENCIES:**

This study was only conducted for 72 hours. According to the EPA memo (October 1994) entitled, "Closure on Nontarget Plant Phytotoxicity Policy Issues", three-day OECD algae studies will be reviewed as Tier I screening studies only.

**E. REVIEWER'S COMMENTS:**

The reviewer's results and those of the study authors' were in agreement with regard to the EC<sub>50</sub> value, but differed with regard to the NOAEC values. The study authors' NOAEC results were more conservative, but the reviewer's analysis was able to provide a probit slope and EC<sub>05</sub> values. The reviewer's EC<sub>05</sub> values and NOAEC results are presented in the Executive Summary and Conclusions sections of this DER. However, the reviewer statistically determined the growth rate NOAEC to be 15.3 mg a.i./L. Upon inspection of the data, the reviewer determined that a visual determination of the NOAEC was a more appropriate estimate. The growth rate NOAEC was visually determined to be 59.1 mg a.i./L.

The reviewer verified the mean-measured concentrations and calculated the percent of nominal concentrations at 0 and 72 hours. The reviewer reports these unrounded values in the tables and statistical verification sections.

In order to determine if the test substance had an algistatic or algicidal effect, recovery was assessed for the highest test level, 120 mg ai/L due to an apparent 50% reduction in cell density. However, actual cell counts from the definitive test showed that cell density in this treatment level were only reduced by 48% and thus the testing was terminated due to not being required by the protocol.

Total organic carbon, particulate matter, and chlorine concentrations of the well water used for the algal medium were not reported.

The in-life portion of the algal toxicity test was conducted between January 4 and January 7, 2008.

**F. CONCLUSIONS:**

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This study is scientifically sound classified as acceptable for a Tier I screening study. No endpoint exhibited inhibition in excess of 50%; the EC<sub>50</sub> value for all endpoints was >120 mg ai/L. The NOAEC for cell density and biomass was determined to be 15.3 mg a.i./L and the NOAEC for growth rate was 59.1 mg ai/L. In addition, cell density and biomass shared the lower (reviewer-calculated) EC<sub>05</sub> estimate of 62 mg ai/L.

Test Organism: *Pseudokirchneriella subcapitata*

Test Type (Flow-through, Static, Static Renewal): Static

**(Reviewer's ECx results and study authors' NOAEC results)**

**Cell Density**

EC<sub>05</sub>: 62 mg ai/L 95% C.I.: 41 to 94 mg ai/L

EC<sub>50</sub>: >120 mg ai/L 95% C.I.: N/A

NOAEC: 15.3 mg ai/L

Probit Slope: 5.35 ± 1.60

**Biomass (Area Under the Growth Curve)**

EC<sub>05</sub>: 62 mg ai/L 95% C.I.: 39 to 100 mg ai/L

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**Growth Rate**

EC<sub>05</sub>: 93 mg ai/L 95% C.I.: 77 to 110 mg ai/L

EC<sub>50</sub>: >120 mg ai/L 95% C.I.: N/A

NOAEC: 59.1 mg ai/L

Probit Slope: 3.98 ± 1.35

Endpoint(s) Affected: Cell density, biomass, and growth rate

**III. REFERENCES:**

Organization for Economic Cooperation and Development. 2006. OECD Guidelines for Testing of Chemicals, 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*. Adopted 23 March 2006.

U.S. Environmental Protection Agency. 1996. Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II*.

ASTM Standard Guide 1218-90E. 1990. Standard Guide for Conducting Static 72-Hour Toxicity Tests with Microalgae. American Society for Testing and Materials. Philadelphia, PA.

SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8.2. Cary, NC, SAS Institute, Inc.

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**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)

File: 0203c Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	8	5	8	0

Calculated Chi-Square goodness of fit test statistic = 7.3034

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)

File: 0203c Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1986.080

W = 0.972

Critical W (P = 0.05) (n = 21) = 0.908

Critical W (P = 0.01) (n = 21) = 0.873

Data PASS normality test at P=0.01 level. Continue analysis.

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)

File: 0203c Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 19.30

Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3  
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.50  
(average df used)

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal  
but do not differ greatly, the Hartley test may still be used

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as an approximate test (average df are used).

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)  
File: 0203c Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----  
Calculated B statistic = 5.23  
Table Chi-square value = 15.09 (alpha = 0.01)  
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.50  
Used for Chi-square table value ==> df (#groups-1) = 5  
-----

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is  
used to calculate the B statistic (see above).

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)  
File: 0203c Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	23664.343	4732.869	35.745
Within (Error)	15	1986.080	132.405	
Total	20	25650.423		

Critical F value = 2.90 (0.05,5,15)  
Since F > Critical F REJECT Ho:All groups equal

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)  
File: 0203c Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	217.467	217.467		
2	7.35	206.200	206.200	1.385	
3	15.3	205.300	205.300	1.495	
4	30.1	190.700	190.700	3.290	*
5	59.1	200.100	200.100	2.134	
6	120	113.567	113.567	12.770	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)



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Aminocyclopyrachlor acid & 72-hr cell density (mg/L)  
File: 0203c Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 2 OF 2		Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL	
1	Neg control	6				
2	7.35	3	21.179	9.7	11.267	
3	15.3	3	21.179	9.7	12.167	
4	30.1	3	21.179	9.7	26.767	
5	59.1	3	21.179	9.7	17.367	
6	120	3	21.179	9.7	103.900	

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)  
File: 0203c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	217.467	217.467	217.467
2	7.35	3	206.200	206.200	206.200
3	15.3	3	205.300	205.300	205.300
4	30.1	3	190.700	190.700	195.400
5	59.1	3	200.100	200.100	195.400
6	120	3	113.567	113.567	113.567

Aminocyclopyrachlor acid & 72-hr cell density (mg/L)  
File: 0203c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	217.467				
7.35	206.200	1.385		1.75	k= 1, v=15
15.3	205.300	1.495		1.84	k= 2, v=15
30.1	195.400	2.712	*	1.87	k= 3, v=15
59.1	195.400	2.712	*	1.88	k= 4, v=15
120	113.567	12.770	*	1.89	k= 5, v=15

s = 11.507

Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds	Std.Err.	Lower Bound
		Lower Upper		/Estimate

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EC5	62.	41.	94.	0.086	0.66
EC10	73.	53.	1.0E+02	0.066	0.73
EC25	94.	80.	1.1E+02	0.034	0.85
EC50	1.3E+02	1.2E+02	1.4E+02	0.016	0.93

Slope = 5.35 Std.Err. = 1.60

!!!Poor fit: p = 0.039 based on DF= 3.0 15.

0203C : Aminocyclopyrachlor acid & 72-hr cell density (mg/L)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	217.	208.	9.91	100.	0.00
7.35	3.00	206.	208.	-1.36	100.	2.02e-09
15.3	3.00	205.	208.	-2.26	100.	4.78e-05
30.1	3.00	191.	207.	-16.8	100.	0.0435
59.1	3.00	200.	199.	0.621	96.1	3.89
120.	3.00	114.	114.	-0.0377	54.7	45.3

!!!Warning: EC50 not bracketed by doses evaluated.

Aminocyclopyrachlor acid & 72-hr biomass; mg/L

File: 0203b Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	7	7	0

Calculated Chi-Square goodness of fit test statistic = 4.3919

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Aminocyclopyrachlor acid & 72-hr biomass; mg/L

File: 0203b Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 52.107

W = 0.978

Critical W (P = 0.05) (n = 21) = 0.908

Critical W (P = 0.01) (n = 21) = 0.873

**Data Evaluation Report on the Acute Toxicity of Aminocyclopyrachlor acid technical to  
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Data PASS normality test at P=0.01 level. Continue analysis.

Aminocyclopyrachlor acid & 72-hr biomass; mg/L  
File: 0203b Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

-----  
Calculated H statistic (max Var/min Var) = 43.82  
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3  
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.50  
(average df used)

-----  
Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal  
but do not differ greatly, the Hartley test may still be used  
as an approximate test (average df are used).

Aminocyclopyrachlor acid & 72-hr biomass; mg/L  
File: 0203b Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----  
Calculated B statistic = 8.86  
Table Chi-square value = 15.09 (alpha = 0.01)  
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.50  
Used for Chi-square table value ==> df (#groups-1) = 5

-----  
Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is  
used to calculate the B statistic (see above).

Aminocyclopyrachlor acid & 72-hr biomass; mg/L  
File: 0203b Transform: NO TRANSFORMATION

ANOVA TABLE

-----  
SOURCE DF SS MS F  
-----  
Between 5 419.436 83.887 24.147  
Within (Error) 15 52.107 3.474  
-----  
Total 20 471.543  
-----

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Critical F value = 2.90 (0.05,5,15)  
Since F > Critical F REJECT Ho:All groups equal

Aminocyclopyrachlor acid & 72-hr biomass; mg/L  
File: 0203b Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 1 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	35.067	35.067		
2	7.35	32.733	32.733	1.770	
3	15.3	33.033	33.033	1.543	
4	30.1	30.833	30.833	3.212	*
5	59.1	32.233	32.233	2.150	
6	120	21.033	21.033	10.648	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

Aminocyclopyrachlor acid & 72-hr biomass; mg/L  
File: 0203b Transform: NO TRANSFORMATION

BONFERRONI T-TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	6			
2	7.35	3	3.431	9.8	2.333
3	15.3	3	3.431	9.8	2.033
4	30.1	3	3.431	9.8	4.233
5	59.1	3	3.431	9.8	2.833
6	120	3	3.431	9.8	14.033

Aminocyclopyrachlor acid & 72-hr biomass; mg/L  
File: 0203b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)			TABLE 1 OF 2		
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	35.067	35.067	35.067
2	7.35	3	32.733	32.733	32.883
3	15.3	3	33.033	33.033	32.883
4	30.1	3	30.833	30.833	31.533
5	59.1	3	32.233	32.233	31.533
6	120	3	21.033	21.033	21.033

# Data Evaluation Report on the Acute Toxicity of Aminocyclopyrachlor acid technical to Algae (*Pseudokirchneriella subcapitata*)

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Aminocyclopyrachlor acid & 72-hr biomass; mg/L  
File: 0203b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)			TABLE 2 OF 2		
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	35.067				
7.35	32.883	1.657		1.75	k= 1, v=15
15.3	32.883	1.657		1.84	k= 2, v=15
30.1	31.533	2.681	*	1.87	k= 3, v=15
59.1	31.533	2.681	*	1.88	k= 4, v=15
120	21.033	10.648	*	1.89	k= 5, v=15

s = 1.864

Note: df used for table values are approximate when v > 20.

## Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	62.	39.	1.0E+02	0.098	0.62
EC10	74.	53.	1.1E+02	0.072	0.71
EC25	1.0E+02	87.	1.2E+02	0.031	0.86
EC50	1.4E+02	1.2E+02	1.7E+02	0.031	0.86

Slope = 4.58 Std.Err. = 1.54

!!!Poor fit: p = 0.039 based on DF= 3.0 15.

0203B : Aminocyclopyrachlor acid & 72-hr biomass; mg/L

## Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	35.1	33.4	1.68	100.	0.00
7.35	3.00	32.7	33.4	-0.658	100.	1.93e-07
15.3	3.00	33.0	33.4	-0.357	100.	0.000469
30.1	3.00	30.8	33.4	-2.52	99.9	0.102
59.1	3.00	32.2	32.0	0.203	95.9	4.07
120.	3.00	21.0	21.0	-0.0157	63.0	37.0

!!!Warning: EC50 not bracketed by doses evaluated.

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
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EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	6	8	0

-----  
Calculated Chi-Square goodness of fit test statistic = 5.7230  
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

-----  
D = 1041.500

W = 0.977

Critical W (P = 0.05) (n = 21) = 0.908  
Critical W (P = 0.01) (n = 21) = 0.873

-----  
Data PASS normality test at P=0.01 level. Continue analysis.

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

-----  
Calculated H statistic (max Var/min Var) = 8.81  
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) =	6,	df (# reps-1) =	3
Actual values ==>	R (# groups) =	6,	df (# avg reps-1) =	2.50
			(average df used)	

-----  
Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal  
but do not differ greatly, the Hartley test may still be used  
as an approximate test (average df are used).

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----  
Calculated B statistic = 4.07  
Table Chi-square value = 15.09 (alpha = 0.01)  
Table Chi-square value = 11.07 (alpha = 0.05)

# Data Evaluation Report on the Acute Toxicity of Aminocyclopyrachlor acid technical to Algae (*Pseudokirchneriella subcapitata*)

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Average df used in calculation ==> df (avg n - 1) = 2.50  
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	18295.452	3659.090	52.700
Within (Error)	15	1041.500	69.433	
Total	20	19336.952		

Critical F value = 2.90 (0.05,5,15)  
Since F > Critical F REJECT Ho:All groups equal

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	747.500	747.500		
2	7.35	740.000	740.000	1.273	
3	15.3	739.667	739.667	1.329	
4	30.1	728.333	728.333	3.253	*
5	59.1	736.000	736.000	1.952	
6	120	657.333	657.333	15.303	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	6			
2	7.35	3	15.337	2.1	7.500
3	15.3	3	15.337	2.1	7.833

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4	30.1	3	15.337	2.1	19.167
5	59.1	3	15.337	2.1	11.500
6	120	3	15.337	2.1	90.167

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	6	747.500	747.500	747.500
2	7.35	3	740.000	740.000	740.000
3	15.3	3	739.667	739.667	739.667
4	30.1	3	728.333	728.333	732.167
5	59.1	3	736.000	736.000	732.167
6	120	3	657.333	657.333	657.333

Aminocyclopyrachlor acid & 72-hr growth rate; mg/L  
File: 0203g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	747.500				
7.35	740.000	1.273		1.75	k= 1, v=15
15.3	739.667	1.329		1.84	k= 2, v=15
30.1	732.167	2.602	*	1.87	k= 3, v=15
59.1	732.167	2.602	*	1.88	k= 4, v=15
120	657.333	15.303	*	1.89	k= 5, v=15

s = 8.333

Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	93.	77.	1.1E+02	0.039	0.83
EC10	1.2E+02	1.1E+02	1.2E+02	0.012	0.94
EC25	1.6E+02	1.3E+02	2.0E+02	0.046	0.80
EC50	2.4E+02	1.5E+02	4.0E+02	0.10	0.61

Slope = 3.98 Std.Err. = 1.35

!!!Poor fit: p = 0.042 based on DF= 3.0 15.

0203G : Aminocyclopyrachlor acid & 72-hr growth rate; mg/L



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PMRA Submission Number {.....}

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Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	747.	741.	6.74	100.	0.00
7.35	3.00	740.	741.	-0.758	100.	7.51e-08
15.3	3.00	740.	741.	-1.09	100.	8.95e-05
30.1	3.00	728.	741.	-12.3	100.	0.0156
59.1	3.00	736.	735.	0.709	99.3	0.738
120.	3.00	657.	657.	-0.0332	88.7	11.3

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.